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PINE TISSUE AFFECTED BY BLISTER RUST DESPITE DIALYSIS BARRIER BETWEEN THE ORGANISMS IN CULTURE

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ABSTRACT

Physical and chemical properties of ribonuclease were altered in pine tissue cultures exposed to the blister rust fungus in a co-culture system that prevented physical penetration of host tissues. Changes were similar to those caused by direct infection of pine stems, pine tissue cultures, and Ribes *leaves. Because they occurred in spite of interposed dialysis membranes, the changes were apparently induced by diffusion of pathotoxins of small molecular weight.*

KEYWORDS: Ribonuclease, white pine, blister rust, pathotoxin, host-parasite interaction, physiology, *Pinus monticola*, *Cronartium ribicola*.

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Demonstrable effects on host ribonuclease (RNase) are induced through direct parasitism by *Cronartium ribicola* J. C. Fisch. on *Ribes nigrum* L. leaves, *Pinus monticola* Dougl. tissue cultures (Harvey and others 1974b), and *P. monticola* seedling stems (Harvey 1977). These and other pathological changes occur in advance of physical penetration of the pine host (Harvey 1977; Robb and others 1974; Harvey and others 1974b). This suggests the presence of diffusible pathotoxins in white pine blister rust infections.

Ribonuclease properties were used herein as tools for demonstrating rust induced host changes in a previously developed co-culture system (Harvey and Grasham 1970). Direct penetration of the host was precluded by interposition of dialysis membranes between the parasite and its pine host.

MATERIALS AND METHODS

Pine tissue explants were removed from seedling stock derived from open-pollinated seed collected in the St. Joe National Forest in north Idaho. Seedlings were grown in nursery beds for ca. 7 years. Explant preparation and culture methods have been described (Harvey and Grasham 1970). Measurements reported here were taken from a clonal culture derived from a single seedling.

Rust inoculum for co-culturing was derived from an axenic culture isolate. This isolate is deposited with the American Type Culture Collection and is designated #PR-91. In all other respects, the co-culture technique was identical to that previously reported.

For this study, the rust was propagated in a basal medium of known composition (Harvey and Grasham 1974a) fortified with 10 mg/l vitamin-free, casein acid hydrolysate as a source of amino acids.

Co-cultures were produced by placing whole, 30-day-old axenic rust cultures onto dialysis membranes (pore size 4.8 micrometer) covering host tissues imbedded in the culture medium (fig. 1). This was done 90 days after initial establishment of the host cultures. Co-cultures were incubated for an additional 150 days (16 at 20° C, 400 fc fluorescent light, and 8h at 5° C, no light).

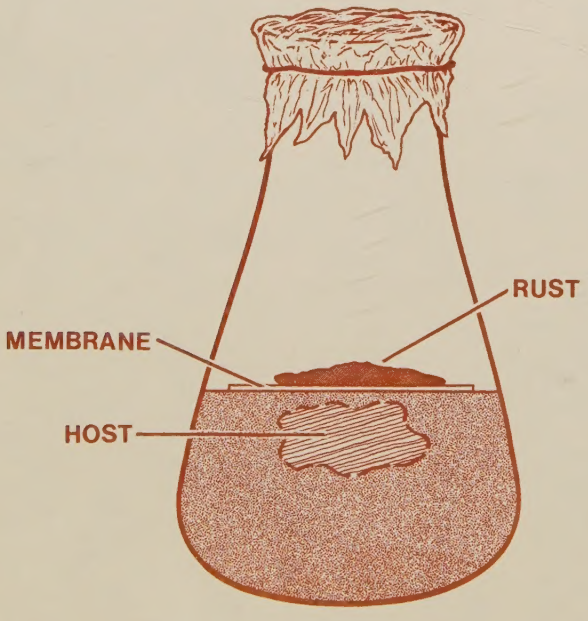

Figure 1.--Diagram of host-rust co-culture system used in this study. Cultured in 125 ml flasks covered with cellophane (Harvey and Grasham 1970).

Host tissues used for extraction were prepared by gently removing whole cultures from the flasks and washing away all adhering agar substrate by rinsing with distilled water. Approximately 2.0 g (one whole, undamaged culture) was extracted immediately after rinsing.

Rust tissues used for extraction were removed from the membrane co-cultures at the time host tissues were prepared. These tissues were rinsed in distilled water, combined with 5-10 identical cultures (minimum of 0.5 g fresh weight) and extracted immediately.

Healthy host tissues were produced exactly the same as rust exposed host tissues except that the membranes were not inoculated. Handling of tissues in preparation for extraction was accomplished with scrupulously cleaned instruments and vessels while wearing disposable plastic gloves. All possible care was exercised to prevent contamination of samples with ribonuclease from any external source.

Enzyme assays were as described by Scrubb and others (1972). One unit of RNase is defined as the quantity catalyzing an increase in A_{260nm} of 1.0 under standard conditions of assay, and specific activity in units/mg protein. Specific activities of other hydrolases are expressed as ΔOD at the appropriate wavelength/mg of protein.

Because the tissues used herein were somewhat different than those for which the measurement methods were developed, all measurements were based on at least three separate extractions of different samples, with minor changes in the extraction procedures. Enzyme assays were also carried out in triplicate, with minor changes in the preparation or assay procedure. The results reported here are based on the optimal enzyme activity achieved.

RESULTS AND DISCUSSION

Although conditions prevailing during extraction procedures and in RNase reaction vessels used in this study were generally unfavorable to hydrolytic activity of deoxyribonuclease (DNase), phosphodiesterase, alkaline phosphatase, and acid phosphatase, their activities were measured. Table 1 summarizes typical values when tissue extracts were assayed under optimum conditions for each. Rates of hydrolytic activity in rust-exposed pine tissues were lower than in tissues not exposed to the rust. This indicates that any increases in RNA hydrolysis products are derived from RNase activity.

Table 1.--*Changes in the activities of enzymes (other than ribonuclease) capable of hydrolyzing the phosphodiesterase bond, from healthy and blister-rust exposed pine tissue cultures*

Tissue	Hydrolytic enzyme ΔOD at appropriate wavelength per mg protein			
	DNase[1]	Acid phosph[2]	Alk phosph	Phospho-diesterase
Healthy (unexposed) pine tissue culture	14.8	23.9	7.7	0.0
Rust-exposed-(5 mo) pine tissue culture	9.4	18.3	3.4	0.0

[1]Deoxyribonuclease.
[2]Phosphatase.

The effects of pH on activity of RNase extracts from various tissues is shown in figure 2. Both rust and rust-exposed pine tissues had higher specific activities at their optimum pH than healthy host tissues. The rust-exposed pine tissue enzyme preparations were also more stable to pH change than those of either rust or healthy host tissue. The pH optimum (4.5) of these preparations were identical for all tissues.

Table 2 shows relative rates of hydrolysis of four radiolabelled homoribopolymers resulting from exposure to RNase preparations from rust, rust exposed pine, and healthy pine tissue cultures. Total activity was highest in rust tissues and lowest in healthy pine tissue. A similar order was shown in figure 2. The ability of pine tissue RNase extracts to break down these substrates changed dramatically when the tissue cultures were exposed, via the dialysis membrane, to the rust fungus. Most striking of these effects was a doubling in the hydrolysis rate of polyadenylic acid and halving the hydrolysis rate of polyuridylic acid. Rust RNase was also highly active against polyadenylic acid.

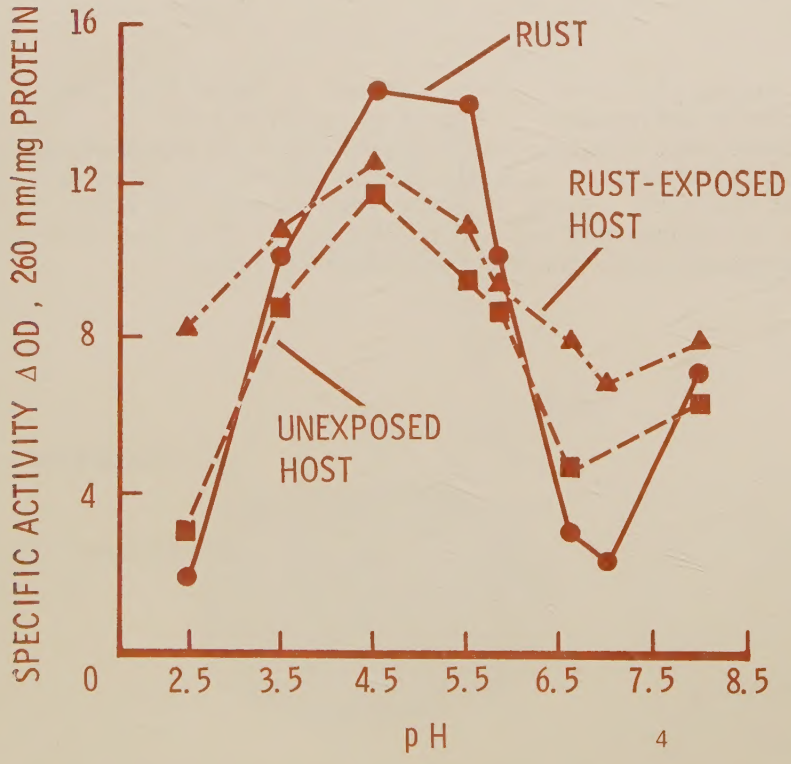

Figure 2.--pH activity curves for RNase extracts of rust, rust-exposed, and healthy pine tissue cultures, after 5 months in co-culture. Assay buffers were: Na_2HPO_4-citric acid, pH 2.5-4.5; KH_2PO_4-NaOH, pH 5.5-7.0; Tris-Hcl, pH 8.0-8.5.

The effect of diethylpyrocarbonate on the activity of RNase extracted from these tissues was also striking. The ability of both rust and healthy host tissue RNase to hydrolyze polyinsinic acid was highly sensitive to the presence of this inhibitor. However, RNase from host tissues that were exposed to the rust was more stable (table 3).

Table 2.--*Hydrolysis of radiolabeled polynucleotides by RNase extracts from blister rust, rust-exposed, and healthy pine tissue cultures*

Tissue	Hydrolysis dpm/g protein x 10^{-3}				
	Poly (A)[1]	Poly (U)	Poly (I)	Poly (C)	Total dpm
Healthy (unexposed) pine tissue culture	504	497	79	3	1,087
Rust-exposed-(5 mo) pine tissue culture	1,100	231	89	1	1,421
Rust mycelium	2,488	679	478	0	3,645

[1]Abbreviations used are: poly (A), poly (U), poly (C), and poly (I), polyadenilic, polyuridilic, polycytidilic, and polyinosinic acids, respectively.

Table 3.--*Diethylpryocarbonate (DEP) inhibition of hydrolysis of ^{14}C-labeled polyinosinic acid by RNase from blister rust, rust-exposed, and healthy pine tissue cultures*

Tissue	Hydrolysis dpm/mg protein		DEP inhibition percent
	-DEP	+DEP	
Healthy (unexposed) pine tissue culture	19,200	1,500	80
Rust-exposed-(5 mo) pine tissue culture	509,000	226,000	67
Rust mycelium	16,000	--	100

Evaluations of temperature stability showed that the highly active RNase extract from rust tissues was highly temperature sentitive, extract from healthy host tissues was relatively stable, and extract from rust exposed host tissues was possibly slightly less stable than from the healthy host (table 4). Heat sensitivity of RNase from blister rust-infected tissues has been reported elsewhere (Harvey 1977; Harvey and others 1974b).

Table 4.--*Thermal stability for RNase from blister rust, rust-exposed, and healthy pine tissue cultures*[1]

Tissue	Specific activity ΔOD 260nm/mg protein		Percentage inhibition after heating
	10 min 0° C	10 min 80° C	
Healthy (unexposed) pine tissue culture	2.9	1.3	55
Rust-exposed-(5 mo) pine tissue culture	3.3	1.6	59
Rust mycelium	3.0	0.2	93

[1] Enzyme extracts were treated by holding at 80° C for 10 min, cooling to 0° C, then assaying for residual activity.

CONCLUSIONS

The results presented herein demonstrate that some of the characteristic changes in host RNase activity resulting from interactions with various obligate parasites (Chakravorty and Shaw 1977; Harvey and others 1974b; Scrubb and others 1972; Reddi 1966; Diener 1961) may also occur when the interaction process is limited to the exchange of small molecular weight metabolites. They confirm that one or more diffusible pathotoxins are produced by the blister rust fungus (Harvey and others 1974b; Robb and others 1974; Harvey 1977) in the presence of its pine host.

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